

# CellTrace™ Violet Cell Proliferation Kit

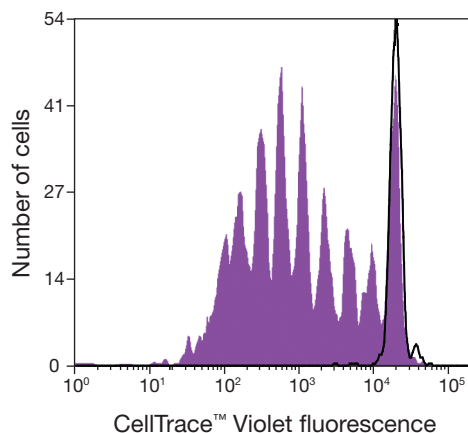
Catalog no. C34557

**Table 1.** Contents and storage information.

Material	Amount	Storage	Stability
CellTrace™ Violet (Component A)	9 vials	<ul style="list-style-type: none"> <li>• ≤-20°C</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	When stored as directed, the product is stable for at least 1 year.
DMSO (Component B)	500 µL	≤-20°C	
<b>Number of reactions:</b> Sufficient material is supplied for 180 reactions, based on the protocol below.			
<b>Approximate fluorescence excitation/emission maxima:</b> CellTrace™ Violet: 405/450 in nm.			

## Introduction

The CellTrace™ Violet Cell Proliferation Kit provides a versatile and well-retained cell tracing reagent in a convenient and easy-to-use form. The kit contains CellTrace™ Violet in nine single-use vials to permit small scale experiments without preparing excess quantities of stock solution. CellTrace™ Violet easily diffuses into cells where it is cleaved by intracellular esterases to yield a highly fluorescent compound. This compound covalently binds to intracellular amines, resulting in stable, well-retained fluorescent staining that can be fixed with aldehyde fixatives. Excess unconjugated reagent passively diffuses to the extracellular medium, where it can be quenched with complete media and washed away.



**Figure 1.** Human peripheral blood lymphocytes were harvested and stained with CellTrace™ Violet. The violet peaks represent successive generations of cells stimulated with mouse anti-human CD3 and Interleukin-2 and grown in culture for 7 days. The peak outlined in black represents cells that were grown in culture for 7 days with no stimulus.

## Before Starting

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### Materials Required but Not Provided

- Cells of interest as a single-cell suspension
- Phosphate-buffered saline (PBS) or similar protein-free buffer
- Culture media compatible with cells of interest
- Flow cytometer with ~405 nm laser and ~450 nm emission filter

### Caution

No data are available addressing the mutagenicity or toxicity of CellTrace™ Violet (Component A). Handle the DMSO dye solution with caution because DMSO is known to facilitate the entry of organic molecules into tissues. Always wear suitable protective clothing, gloves, and eye/face protection when handling this reagent. Dispose of the reagents in compliance with all pertaining local regulations.

### Storage and Handling

Upon receipt, store the kit components desiccated at  $\leq -20^{\circ}\text{C}$  until required for use. Avoid repeated freezing and thawing of CellTrace™ Violet. Before opening the vial, allow the product to warm to room temperature. When stored properly, DMSO and solid CellTrace™ Violet are stable for at least one year. Use the DMSO solutions of the reagent the same day of preparation.

## Experimental Protocols

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### Labeling Cells for Analysis in Flow Cytometry

Follow the guidelines below for labeling cells with CellTrace™ Violet for analysis using flow cytometry.

- The following methods have been optimized for monitoring cell proliferation in populations of human B and T lymphocytes.
- In other cell types and applications, determine the optimal working concentration of CellTrace™ Violet by titrating the reagent. You may further dilute a portion of the stock solution in DMSO prior to use for this purpose.
- Use working concentrations of CellTrace™ Violet in the range of 1–10  $\mu\text{M}$ .
- Start with a single cell suspension for uniform cell labeling.
- To ensure appropriate instrument setup, include an unstimulated control in proliferation experiments using CellTrace™ Violet.

### Standard Method for Labeling Cells in Suspension

The following protocol has been optimized for cell concentrations up to  $10^6$  cells/mL. You may need to increase the dye concentration for samples with  $>10^6$  cells/mL.

- 1.1** Prepare a 5 mM CellTrace™ Violet stock solution immediately prior to use by dissolving the contents of one vial of CellTrace™ Violet (Component A) in 20  $\mu\text{L}$  of DMSO (Component B).
- 1.2** Add 1  $\mu\text{L}$  of 5 mM CellTrace™ Violet stock solution in DMSO (prepared in Step 1.1) to each mL of cell suspension for a final working concentration of 5  $\mu\text{M}$ .
- 1.3** Incubate the cells for 20 minutes at  $37^{\circ}\text{C}$ , protected from light.

- 1.4 Quench any unbound dye by adding 5 times the original staining volume of complete culture medium to the cells and incubating them for 5 minutes.
- 1.5 Pellet the cells by centrifugation and resuspend them in fresh pre-warmed complete culture medium.
- 1.6 Incubate the cells for at least 10 minutes before analysis to allow the CellTrace™ Violet to undergo acetate hydrolysis.

**Alternate Method for Labeling  
Cells in Suspension**

The following protocol has been optimized for cell concentrations up to  $10^6$  cells/mL. You may need to increase the dye concentration for samples with  $>10^6$  cells/mL.

- 2.1 Prepare a 5 mM CellTrace™ Violet stock solution immediately prior to use by dissolving the contents of one vial of CellTrace™ Violet (Component A) in 20  $\mu$ L of DMSO (Component B).
- 2.2 Pellet the cells by centrifugation and remove the supernatant.
- 2.3 Dilute the 5 mM CellTrace™ Violet DMSO stock solution in pre-warmed (37°C) phosphate-buffered saline (PBS) or other protein-free buffer to the desired working concentration (1–10  $\mu$ M).
- 2.4 Gently resuspend the cells in PBS containing the dye (prepared in Step 2.1).
- 2.5 Incubate the cells for 20 minutes at 37°C, protected from light.
- 2.6 Quench any unbound dye by adding 5 times the original staining volume of complete culture medium to the cells and incubating them for 5 minutes.
- 2.7 Pellet the cells by centrifugation and resuspend them in fresh pre-warmed complete culture medium.
- 2.8 Incubate the cells for at least 10 minutes before analysis to allow the CellTrace™ Violet to undergo acetate hydrolysis.

**Alternate Method for Labeling  
Adherent Cells**

- 3.1 Prepare a 5 mM CellTrace™ Violet stock solution immediately prior to use by dissolving the contents of one vial of CellTrace™ Violet (Component A) in 20  $\mu$ L of DMSO (Component B).
- 3.2 Grow the cells to the desired density on coverslips or flasks filled with the appropriate culture medium.
- 3.3 Dilute the 5 mM CellTrace™ Violet DMSO stock solution in pre-warmed (37°C) phosphate-buffered saline (PBS) or other protein-free buffer to the desired working concentration (1–10  $\mu$ M). This is the loading solution.
- 3.4 Remove the culture medium from the cells and replace it with the loading solution (prepared in Step 3.3).
- 3.5 Incubate the cells for 20 minutes at 37°C.
- 3.6 Remove the loading solution, wash the cells twice with fresh, pre-warmed complete culture medium, and replace with fresh, pre-warmed complete culture medium.
- 3.7 Incubate the cells for at least 10 minutes to allow the CellTrace™ Violet to undergo acetate hydrolysis.

## Optional Fixation and Permeabilization

- 4.1 Label the cells with CellTrace™ Violet according to one of the protocols listed above.
- 4.2 Before fixation, wash and resuspend the cells with PBS or other protein-free buffer.
- 4.3 Fix the cells for 15 – 20 minutes at room temperature using an aldehyde-based fixative, protected from light.
- 4.4 Wash the cells with PBS.
- 4.5 If needed, permeabilize the cells using any appropriate protocol.
- 4.6 Following permeabilization, wash the cells with PBS.
- 4.7 Resuspend the cells in PBS prior to acquisition.

## Combining CellTrace™ Violet with other Fluorescent Markers

- 5.1 Label the cells with CellTrace™ Violet according to one of the protocols listed above.
- 5.2 Resuspend the cells in a buffer appropriate for the subsequent staining applications (see below).
- 5.3 Apply stains for immunophenotyping, DNA content, apoptosis, or other markers as recommended for each stain.

## References

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1. J Cell Biol 101, 610 (1985); 2. J Cell Biol 103, 2649 (1986); 3. J Immunol Methods 171, 131 (1994); 4. J Exp Med 184, 277 (1996); 5. J Immunol Methods 133, 87 (1990); 6. Transplant Proc 24, 2820 (1992); 7. Current Protocols in Cytometry, J. P. Robinson, Ed., (1998) pp 9.11.1-9.11.9.

## Product List **Current prices may be obtained from our website or from our Customer Service Department.**

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Cat. no.	Product Name	Unit Size
C34557	CellTrace™ Violet Cell Proliferation Kit *for flow cytometry* .....	1 kit
<b>Related Products</b>		
C34554	CellTrace™ CFSE Cell Proliferation Kit *for flow cytometry* .....	1 kit
MHCD0300	Purified Mouse anti-human CD3 .....	500 µL
PHC0026	Recombinant Human Interleukin-2 .....	10 µg
111.31D	DynaBeads® CD3/CD28 T Cell Expander .....	2 mL
08-0022SA	OpTmizer™ T-Cell Expansion SFM .....	500 mL
10439-016	Fetal Bovine Serum, ES Cell-Qualified .....	100 mL
14190-136	Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X), liquid .....	1000 mL

## Contact Information

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